Public health and public law issues for the toxicological risk assessment of chemical mixtures

Silvio Roberto Vinceti[1], Tommaso Filippini[2]

AFFILIATION
1 Department of Law, University of Modena and Reggio Emilia, Modena, Italy
2 Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

CORRESPONDENCE TO
Tommaso Filippini. Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41125, Modena, Italy.
E-mail: tommaso.filippini@unimore.it ORCID ID: https://orcid.org/0000-0003-2100-0344

ABSTRACT

Humans are simultaneously exposed to a near-infinite number of substances of both nutritional and toxicological interest. While traditional risk assessment has been based on the evaluation of effects of single substances, real-life exposure is characterized by their combination into chemical mixtures. As consequence, the need to focus on substance interactions is of considerable relevance. From the public health point of view, factors able to modulate the kinetic and toxicity of chemicals need to be included in the assessment of health effects. Several European projects including SOLUTIONS, EuroMix, ECD-MixRisk and HBM4EU have been implemented aiming at the characterization of hazardous properties of chemical mixtures and the development of new approaches and tools for their risk assessment, including advanced statistical methodologies. From the public law point of view, the legislative status of mixtures is outlined in a still general way, with a specific focus on a few substances only, e.g. dietary exposure to pesticide residues. Although some advances have been made with the ongoing revision of CLP Regulation specifically focusing also on chemical mixtures, identification of mixtures and the regulation of their use raise relevant and challenging issues to be timely addressed within a collaboration of specialists from toxicology, public health, and public law. Moreover, given the transnational scope of the problem, risk assessment of chemical mixtures presents a valuable occasion for a comparative law approach.

INTRODUCTION
Mixtures, public health and toxicology

Humans are exposed to several substances of nutritional and toxicological relevance from environmental sources including food, air, and drinking water. As a consequence, our environment can be considered a source of a near-infinite number of chemical mixtures[1]. There is growing evidence that a thorough risk assessment should not only focus on the independent effects of one chemical but should take into consideration the joint exposure to a complex mixture of substances of nutritional and toxicological relevance to which living organisms are exposed in real life[2]. However, the current safety risk assessment is primarily based on understanding the effects of single substances rather than their real-life combinations into mixtures[3], thus probably overlooking their combined effects[4].

The human risk assessment of exposure traditionally follows the classical approach of estimating the external exposure and comparing it with health-based monitoring guidance values, generally based on evaluation of chemical concentrations in biomarkers of on-going exposure or body burden[5]. Many factors can modulate the kinetics and toxicity of chemicals in addition to their overall exposure, and consequently have an impact on the health effects

associated with their exposure[6], beyond their actual internal dose resulting from all sources and pathways (oral, dermal, and inhalation)[7,8]. Evidence supporting that nutritional and toxic chemicals can modulate each other, their kinetics, and biological activity, further implies that interactions occurring within chemical mixtures have a potentially strong impact on health outcomes and risk assessment[6].

Studies investigating the simultaneous exposure to multiple chemicals should therefore assess the interactions between these factors – which are not detected when working with one substance at a time – and bear in mind that human populations are almost always exposed to a rather large number of complex substances simultaneously. This is the reason for the growing awareness and debate over the relevance of chemical mixtures in risk assessment[2,9]. At European level, the Joint Research Centre (JRC) of the European Commission has started to investigate the progress in considering combined exposures to multiple chemicals in order to help translate best science into best risk assessment practice[10]. For this purpose, a 2018 JRC policy brief entitled 'Something from nothing? Ensuring the safety of chemical mixtures' confronted various issues on this topic, including the specific challenges and activities to be carried out in Europe[10]. These include the assessment of combined exposure, especially the composition of unintentional mixtures, of combined effects through smart strategies and innovative computational tools, and of combined risks focusing among the large number of possible mixtures on priority mixtures of particular concern[10]. More recently, supranational agencies for risk assessment such as the European Food Safety Authority (EFSA) have highlighted and emphasized the issue of chemical mixture evaluation in the 2020–2022 programming document[11]. Therefore, there is clearly the need to increase our knowledge about mixture effects, designing a strategy capable to assess multi-chemical and multi-pathway exposures of humans and their implication on health. In addition to envisaging a stronger effort in addressing risk assessment of chemical mixtures, the aforementioned EFSA document explicitly states that: 'The integration of New Approach Methodologies (NAMs) in EFSA risk assessments will cover three complementary goals, reduction of animal testing, filling hazard information gaps for data-poor chemicals, and last but not least, moving towards more informative risk assessments, through the integration of existing (human/animal) data and NAMs, for a better mechanistic understanding of the biological interactions that lead to the hazards and risks of chemicals, both in isolation and in chemical mixtures'.

METHODOLOGICAL APPROACH
Mixture investigations: EU projects and advanced statistical tools
While several approaches have been considered and variously combined to investigate chemical mixtures[12-15], few studies have directly addressed the specific challenges

involved in the formal analysis of synergistic and antagonistic interactions within components of a mixture[16-19]. Together with the leverage and development of statistical techniques for addressing the underlying research questions, studies specifically focusing on assessing complex interactions have the potential to improve the risk assessment of single chemicals (including nutrients) within a holistic approach. To this purpose, the European Commission has promoted the implementation of studies and research consortia that assess chemical mixtures in both the environment (e.g. SOLUTIONS project[20]) and human health (e.g. EuroMix[21] and ECD-MixRisk[22]). With regard to the latter category, a very promising project is the European Human Biomonitoring Initiative for Europe (HBM4EU)[23] where the assessment of several chemicals has been accomplished through a specific framework that singles out priority substance group mixtures, including the collection of information on their characterization and hazardous properties, and evaluates existing evidence of human exposure to mixtures in Europe[24]. Similarly, the epidemiologic literature is now beginning to address lower-order interactions between components of chemical mixtures[25], while statistical methodologies that use machine learning techniques for evaluating a large number of exposures can be potentially used to detect high-order interactions[16,19,26-29]. For example, several procedures have been recently developed to assess the overall effect of the mixture, such as the Weighted Quantile Sum (WQS) regression, elastic net regression, and Bayesian Kernel Machine Regression (BKMR), elastic net regression for interaction (INTRANET), the deletion/substitution/addition (DSA) algorithm, and boosted regression trees, some of them taking also into account the individual dose-response associations with the outcome of interest[13,14,16,30-34]. These innovative approaches that assess chemical interactions have also taken into consideration the similarity of biological effects in chemicals that differ only in their potencies and in their ability to act through different modes of action or at different sites in the human body – possibly also as a consequence of their chemical form – a feature that adds further complexity to their effects and interactions[35].

Mixtures and public law
The assessment and management of chemical mixtures is only partly covered by current legislation, which unfortunately focuses on single substances. In addition, the legislative status of mixtures varies greatly depending on the different types alternatively considered.

Intentional mixtures
Formulated products marketed as such and for this reason covered by the Chemical Labelling and Packaging-CLP requirements[36]. For intentional mixtures, the composition is known and assessments are based on the properties of the individual constituents and tests are generally carried out on the entire product.

Unintentional mixtures

Mixtures originated from a single source where: 1) the composition is known and therefore assessment can be made based on knowledge of the constituents; and 2) the composition is unknown, in this case the assessment can in principle be based on tests carried out on the whole mixture or based on the single substances.

Coincidental mixtures

Mixtures of chemicals originated from multiple sources and through multiple routes.

Regulations

In the context of the REACH Regulation[37], guidance has been developed concerning the assessment of multiple sources of exposure to a single substance and in specific cases to the assessment of several closely related and similarly acting substances, such as different salts of the same metal or a number of closely related derivatives of organic substances (see for example ECHA 2016, section E.3.5). While this gives some scope to assess possible adverse effects associated with known combinations, it does not address possible concerns associated with exposure to unknown mixtures. Another example of regulation of mixtures is available for pesticides with the Regulation 396/2005/EC[38] on maximum residue levels of pesticides in either food and feed of plant and animal origin with the subsequent implementation of several activities of the European Food Safety Authority, including Scientific Opinions on mixture pesticides exposure[39,40].

DISCUSSION

Despite these efforts, a substantial lack of legislation can be still noted[21], though improvements are in progress as recently demonstrated by the public consultation to update of the CLP legislation[41], focusing one of the key topics on the importance to take into account the effects of mixtures of chemicals[42-44] and highlight the challenges they pose to both risk assessors and risk managers, i.e. to both toxicology and public law. From a comparative law perspective, moreover, the European legal framework for combined exposures' risk assessment is considered somehow underdeveloped when compared to those in place in the United States or Canada[21].

The very American experience, however, remind us that the legal problems posed by chemical mixtures and their risk assessment are common throughout the world and that international solutions could better address them. More than forty years have passed since the controversial 'Benzene case' of July 1980, where a 5-to-4 US Supreme Court ruled that the requirement of health standards 'reasonably necessary or appropriate to provide safe or healthful employment and places of employment' was not so dire as to demand that employers apply 'the lowest technologically feasible level that will not impair the viability of the industries regulated'[45].

CONCLUSIONS

To prohibit exposure at a certain level, regulatory agencies must demonstrate a 'significant risk'[46]. Forty years after the *Benzene decision*, there is growing awareness in the US about the need to address the ruling and somehow revise it[47]. For this reason, an optimal solution for both European and Non-European agencies is not simply to emulate foreign arrangement, but rather to work together and elaborate standards and common approaches that could lead to a shared legal framework for chemical mixtures[21,48].

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